

also seen at  $\sim 525$  nm, but the position of this weak absorption is difficult to locate.

A similar procedure was used for the neopentylchromium cation, preparing the hydroperoxide from 2,4,4-trimethyl-2-pentanol and hydrogen peroxide. The alcohol was prepared from 2,4,4-trimethyl-2-pentanone and  $\text{CH}_3\text{MgBr}$ , followed by hydrolysis in an ice-sulfuric acid mixture. The neopentylchromium complex was isolated as described for the ethyl derivative; absorption maxima were seen at 387 (204) and 289 (840).

Solutions of  $\text{Cr}(\text{ClO}_4)_2$  were prepared under nitrogen by reduction of  $\text{Cr}(\text{ClO}_4)_3$  with amalgamated zinc.

The chromium concentrations were analyzed spectrophotometrically after oxidation to chromate in alkaline peroxide; at  $\lambda$  372 nm,  $\epsilon = 4830 \text{ M}^{-1} \text{ cm}^{-1}$ . The concentration of  $\text{Br}_2$  in stock solutions was determined spectrophotometrically and by volumetric analysis. The molar absorptivity of  $\text{Br}_2$  at 452 nm is

$103 \text{ M}^{-1} \text{ cm}^{-1}$  and provides a convenient analysis.<sup>32</sup> Solutions were also analyzed by adding aliquots to an excess of sodium iodide and titrating the liberated iodine with sodium thiosulfate.

**Kinetic and Stoichiometric Measurements.** The slower reactions were followed using a Cary Model 12 or 14 spectrophotometer, the latter equipped with a 0–0.1 absorbance scale. The faster reactions were studied using a Durrum stopped-flow spectrophotometer.

The mass spectra<sup>33</sup> of the volatile products were obtained on samples from experiments carried out in which the reactants were contained in a two-bulb assembly.

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(33) We are indebted to Mr. G. D. Flesch of the Ames Laboratory for his help in making these measurements.

## Coordination Chemistry of Sodium and Potassium Complexation with Macrocyclic Polyethers

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**Abstract:** Isolation and paper chromatographic solution studies of MX (where  $\text{M}^+ = \text{Na}^+$  or  $\text{K}^+$ ;  $\text{X}^- = \text{Cl}^-$ ,  $\text{Br}^-$ ,  $\text{I}^-$ , or  $\text{NCS}^-$ ) complexes with macrocyclic polyethers (general abbreviation R), benzo-15-crown-5 (I), dibenzo-18-crown-6 (II), dibenzo-24-crown-8 (III), and dibenzo-30-crown-10 (IV), were carried out. Competitive isolation of R–MX complexes from ethanol has been investigated for  $\text{X}^- = \text{I}^-$  and  $\text{NCS}^-$ . Based essentially on information from ir spectra and X-ray molecular structure, the structures of most of these complexes are discussed. Isolation of the R–ML (where  $\text{L}^-$  is an organic anion obtained by the deprotonation of 2-nitrophenol (H1), 4-nitrophenol (H2), 2,4-dinitrophenol (H3), 2,4,6-trinitrophenol (H4), 2-hydroxybenzoic acid (H6), 2,6-dihydroxybenzoic acid (H6), 2-nitrobenzoic acid (H7), or 2-aminobenzoic acid (H8)) complexes is also reported. Potassium complexes of the types  $[(\text{I})_2\text{-K}]^+\text{L}^-$ ,  $[(\text{I})_2\text{-K}]^+[\text{L},\text{aq}]^-$ ,  $[(\text{I})_2\text{-K}]^+[\text{L},\text{HL}]^-$ , and  $[(\text{I})_2\text{-K}]^+[\text{L},(\text{HL})_2]^-$  are isolated. The relevance of such conjugated anions to natural systems is discussed.

Macrocyclic polyethers (Figure 1, general abbreviation R) are polydentate ligands of flexible conformation which interact with alkali metal ions to form lipid-soluble cations.<sup>1,2</sup> The metal–ligand interactions are of the ion-dipole type and basically controlled by the fit of the metal ion into the “hole” of R. Different workers have measured these forces in diverse media spectrophotometrically,<sup>3–5</sup> calorimetrically,<sup>6</sup> and electrometrically.<sup>7,8</sup>

From different R and alkali metal salts (MX), 1:1, 3:2, and 2:1 (R:MX) complexes have been isolated using stoichiometric and excess R,<sup>1,2</sup> and single crystal X-ray structural analysis in many cases has been accomplished by Truter, *et al.*,<sup>9–13</sup> Dunitz, *et al.*,<sup>14</sup> and Groth.<sup>15</sup>

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The late Professor Sir Ronald Nyholm, however, realized the importance of detailed chemical investigations of R–MX complexes to see whether by using R as the models for the transport mediators of the cell membrane it is possible to find out the role played by the coordination chemistry of sodium and potassium in the preferential uptake of potassium over sodium in natural systems. This led us to undertake the preliminary isolation studies<sup>16</sup> of the complexes using R of different size and flexibility (I–IV, Figure 1) with MX ( $\text{M}^+ = \text{Na}^+$  or  $\text{K}^+$  and  $\text{X}^- = \text{Br}^-$ ,  $\text{I}^-$ , or  $\text{CNS}^-$ ) and ML (where  $\text{L}^-$  is a chelating organic anion obtained from the deprotonation of any of 2-nitrophenol (H1), 2,4-dinitrophenol (H3), 2-hydroxybenzoic acid (H5), and 2-nitrobenzoic acid (H7)).

In the present investigations the range of experiments has been extended to include  $\text{Cl}^-$  for  $\text{X}^-$  and anions of 4-nitrophenol (H2), 2,4,6-trinitrophenol (H4), 2,6-dihydroxybenzoic acid (H6), and 2-aminobenzoic acid (H8) for  $\text{L}^-$ . Synthesis of complexes from media con-

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(12) P. R. Mallinson and M. R. Truter, *J. Chem. Soc.*, 1818 (1972).

(13) D. Bright and M. R. Truter, *J. Chem. Soc.*, 1544 (1970).

(14) Quoted by M. R. Truter, *Struct. Bonding (Berlin)*, 1973.

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taining free HL and water has been attempted successfully. For each R, competitive isolation of sodium and potassium complexes from ethanol was studied using metal iodides and thiocyanates.

Since in natural systems the complexation of the metal ion by the transport carrier has to take place against the competing effect of diverse ligands,  $I_1$ ,  $I_2$ , . . . , present in the bathing media for the membrane, the next phase of the work will involve coordination chemistry studies of sodium and potassium with model ligands for the natural carriers against the competing effect of  $I_1$ ,  $I_2$ , . . . . Before this type of the work is undertaken an assessment of the interaction capability of various  $I_1$ ,  $I_2$ , . . . with these cations in solution should be made. Paper chromatography is apparently a handy tool for this type of mass scale work and is expected to classify various competing ligands into "sodiumphilic" and "potassiumphilic" groups. In this work the technique is standardized using R with MX because data on solution stabilities of such systems, as determined by other techniques,<sup>8</sup> are available for comparison. Ethanol, in which comparison of the metal-ligand interactions was to be made, was used as a developing solvent.

The isolated new phase was characterized as a complex when (i) found to melt at a temperature higher than the melting point of the free R and (ii) did not absorb at the characteristic ir frequency of R (980, 995, 958, and 961  $\text{cm}^{-1}$  for I-IV, respectively). The composition of the complexes was based upon elemental analysis and supported by its ir spectrum to determine solvation.

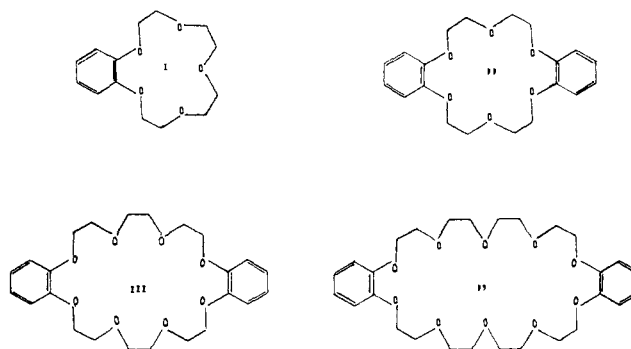
## Experimental Section

**Preparation and Purification of R.** Polyethers used for this work were synthesized following Pedersen's method.<sup>1</sup> The purification and separation of ligands I and IV, both of which are produced in the same reaction, was accomplished as follows.

The mixture of I and IV was dissolved in methanol (10 ml for each gram of the solid) with the help of lithium thiocyanate, 0.1 g for each gram of the polyether mixture. The solution was diluted to twice the volume with distilled water and refrigerated to crystallize out IV. Ligand I stays in solution as a lithium complex. Ligand IV is filtered out and the filtrate was again diluted to twice the volume with water and chilled to ensure the complete elimination of IV. The filtrate was acidified with an amount of dilute sulfuric acid equivalent to the lithium salt used and evaporated to dryness in a rotary evaporator. The residue was treated with ethanol to dissolve I while lithium sulfate was left undissolved. The filtered solution was evaporated to dryness and I was purified employing Parsons method which involves the distillation of the crude polyether at about 1-mm pressure at nearly 200°.

**Synthesis of Complexes from MX.** Complexes were synthesized by dissolving the required amounts of R and MX in any polar organic media whichever can dissolve the reactants, usually a 1:1 mixture of methanol-ethanol. The solution was slowly evaporated at room temperature until the crystals appeared or all the solvent was driven off. In the latter case the product was examined under the microscope and separated from the reactants, if any. In the former case the homogeneity was ascertained under the microscope and the filtrate was again subjected to crystallization or evaporation to dryness to ascertain the presence of any second new product.

**Synthesis of ML Compounds and Their Complexes with R.** ML compounds were prepared in ethanol by the 1:1 reactions of sodium or potassium hydroxide with HL.<sup>17</sup> The crude ML species were crystallized from absolute ethanol. To obtain the crystalline complexes of ML with R, the solution of a 1:1 or 1:2 reaction mixture of the two in ethanol was cooled at room temperature or in the



**Figure 1.** Showing macrocyclic polyethers: I, benzo-15-crown-5; II, dibenzo-18-crown-6; III, dibenzo-24-crown-8; IV, dibenzo-30-crown-10. All named informally after Pedersen.<sup>1</sup>

refrigerator while keeping the beaker open to allow absorption of the necessary amount of water from the atmosphere. The rest of the procedure was as mentioned for MX complexes.

**Synthesis of Complexes Containing  $[L_2HL]^-$  and  $[L_2(HL)_2]^-$  Anions.** The synthesis of such complexes was tried using different proportions of R, ML, and HL from absolute ethanol (EtOH) and 10% aqueous ethanol (EtOH-H<sub>2</sub>O). As in R-MX (or -ML) reaction mixtures the filtrate after the removal of one batch of product was examined for presence of the second.

Melting points were recorded using a Gallankamp micromelting point apparatus and infrared spectra using a Perkin-Elmer spectrophotometer, Model 137. Alkali metal determinations were carried out photometrically using Toshniwal flame photometer, Model Cat. No. RL-01. Carbon, hydrogen, and nitrogen analyses in a few cases were carried out at the Microanalytical laboratories of University College, London, and the rest using a GDR (German) microanalyzer in our department.

**Paper Chromatography.** Paper chromatographic developments were carried out on Whatman No. 1 disks of 15-cm diameter employing the radial method.<sup>18</sup> Ethanol, in which metal-ligand interactions were to be compared for different cases, was used as a developer (irrigating solvent) without expelling the normal aqueous contents of the supporting media (paper). Slow rate of development, 1-2 cm/hr, was maintained using a narrow triangular wick<sup>19</sup> for absorbing the solvent. Systems to be compared were chromatographed simultaneously by spotting as described.<sup>20</sup> Only salt on the paper was detected using the reagent-impregnated pad technique:<sup>21</sup> iodide with 0.1% cupric sulfate which contained 1.0% each of starch and sulfuric acid, thiocyanate with 1.0% aqueous ferric chloride, and the slow running bromide using iodine vapors. The colors obtained are blue, red, and brown, respectively.

## Results and Discussion

For inorganic salts, MX, Table I contains the summary of results where italic values are the results re-

**Table I.** Showing the Polyether-MX Complexes Stoichiometries and the Degree of Hydration<sup>a</sup>

Poly-ether	Sodium			Potassium		
	I <sup>-</sup>	Br <sup>-</sup>	NCS <sup>-</sup>	I <sup>-</sup>	Br <sup>-</sup>	NCS <sup>-</sup>
I	<i>1:1</i> aq	1:1 aq	<i>1:1</i>	2:1	2:1 aq	2:1 aq
II	1:1, 2aq	<i>1:1</i> , 2aq	1:1	1:1 aq	<i>1:1</i> aq	<i>1:1</i>
III	1:1 aq ?	1:1 aq ?	1:1 aq	1:2 aq ?	1:2 aq ?	1:2
IV	1:2 aq	...	1:2 aq	<i>1:1</i>	...	1:1 aq

<sup>a</sup> (· · ·) shows that complex is not isolable and the interrogation denotes that isolation is not reproducible, sometimes.

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Table II. Showing the Details with Regard to the New Complexes with Polyethers

Compound <sup>a</sup>	Color of crystals	Behavior on heating <sup>a</sup>	Reaction mixture best suited for isolation <sup>b</sup> R:ML, media	Elemental analysis, %							
				Calcd				Found			
				C	H	N	M	C	H	N	M
[I-Na] <sup>+1-</sup> <sup>e</sup>	Yellow	105 m	1:1, A	55.94	5.59	3.26	5.36	55.80	5.40	3.31	5.45
[I-Na] <sup>+1-</sup> , ac <sup>f</sup>	Orange	85-115 m	2:1, A	56.67	6.16	2.87	4.72	56.72	5.95	2.95	4.90
[I-Na] <sup>+3-</sup>	Yellow	90-95 m	1:1, A	50.63	4.85	5.90	4.85	49.97	5.01	5.81	4.98
[I-Na] <sup>+4-</sup>	Yellow	153m	1:1, A, Aa, E, or Ea	46.25	4.24	8.08	4.43	46.00	4.32	8.17	4.35
[I-Na] <sup>+7-</sup>	Yellowish white	136-39 m	1:1, A	55.14	5.24	3.63	5.03	55.22	5.32	3.65	4.95
[I-Na] <sup>+8-</sup>	Off white	128-33 m	1:1, A	59.01	6.09	3.28	5.38	58.92	5.98	3.31	5.45
[(1) <sub>2</sub> -K] <sup>+1-</sup> , aq <sup>e</sup>	Orange	138 m	2:1, Aa	55.88	6.29	1.90	5.33	55.55	6.37	1.87	5.35
[(1) <sub>2</sub> -K] <sup>+3-</sup> , aq	Yellow	105-10 t, 135 m	2:1, Aa	52.57	5.79	3.60	5.02	52.62	5.81	3.65	5.11
[(1) <sub>2</sub> -K] <sup>+4-</sup>	Yellow	137 m, 173 m	2:1, A, Aa, E, or Ea	50.80	5.35	5.23	5.85	50.77	5.20	5.18	4.70
[(1) <sub>2</sub> -K] <sup>+5-</sup> , aq	Colorless	119 m, 129 m	2:1, Aa	57.53	6.43		5.34	57.22	6.31		5.50
[(1) <sub>2</sub> -K] <sup>+6-</sup>	Off white	145-47 m	2:1, A, Aa, E, or Ea	57.69	6.18		5.35	57.72	6.15		5.35
[(1) <sub>2</sub> -K] <sup>+7-</sup> , aq	Yellowish white	98 m, 218 m	2:1, Aa	53.75	6.06	1.84	5.13	53.80	6.11	1.81	5.18
[II-Na] <sup>+1-</sup> , aq	Orange-yellow	162-64 m,d	1:1, Aa	57.98	4.80	2.59	4.26	57.50	4.87	2.58	4.32
[II-Na] <sup>+3-</sup> , aq	Yellow	115-125 m	1:1, Aa	53.42	4.28	4.78	3.93	53.22	4.38	4.75	3.90
[II-Na] <sup>+5-</sup> , aq	Colorless	120-125 m,d	1:1, Aa	60.22	5.01		4.27	59.97	5.19		4.35
[II-K] <sup>+1-</sup> , aq	Yellow	112-114 m	1:1, Aa	56.30	4.68	2.51	7.03	56.01	4.74	2.47	6.92
[II-K] <sup>+5-</sup> , aq	Colorless	120 m	1:1, Aa	58.48	4.87		7.04	58.12	5.01		7.14

## Potassium Compounds Obtained from R, ML, and HL Reaction Mixtures where R is I

Compound	Color	Melting point	R:KL:HL	Elemental analysis, %							
				C	H	N	M	C	H	N	M
[(1) <sub>2</sub> -K] <sup>+1,H1</sup> -	Yellow	120-125 m	2:1:1, E or A	56.33	6.76	3.24	4.57	56.52	5.78	3.32	4.50
[(1) <sub>2</sub> -K] <sup>+2,H2</sup> -	Yellow	135-140 m	2:1:1, E or A	56.33	6.76	3.24	4.57	56.68	5.84	3.47	4.59
[(1) <sub>2</sub> -K] <sup>+3,H3</sup> -	Yellow	98-100 m	2:1:1, E or A	50.95	4.98	5.94	4.14	51.36	5.23	5.76	4.25
[(1) <sub>2</sub> -K] <sup>+3,(H3)<sub>2</sub></sup> -	Yellow	118-122 m	2:1:2, Ea	49.00	4.52	7.46	3.45	48.07	4.61	7.04	3.62
[(1) <sub>2</sub> -K] <sup>+4,H4</sup> -	Orange-yellow	176 m	2:1:1, E or A	46.54	4.55	8.13	3.77	46.71	4.38	7.59	3.91
[(1) <sub>2</sub> -K] <sup>+5,H5</sup> -	Colorless	114 m	2:1:1, E or A	59.22	6.02		4.70	59.36	6.02		4.63
[(1) <sub>2</sub> -K] <sup>+5,(H5)<sub>2</sub></sup> -	Colorless <sup>d</sup>	89 m	2:1:2, Ea	59.50	5.80		3.95	59.40	5.77		3.90
[(1) <sub>2</sub> -K] <sup>+6,H6</sup> -	Colorless	124-126 m	2:1:1, E or A	57.14	5.78		4.42	57.00	5.85		4.50
[(1) <sub>2</sub> -K] <sup>+7,H7</sup> -	Yellowish white	118-125 m	2:1:1, E or A	55.50	5.36	3.08	4.29	55.29	5.35	2.91	4.11
[(1) <sub>2</sub> -K] <sup>+7,(H7)<sub>2</sub></sup> -	Yellowish <sup>a</sup>	96 m	2:1:1, Ea	54.69	5.02	3.91	3.63	54.99	5.15	4.09	3.55
[(1) <sub>2</sub> -K] <sup>+8,H8</sup> -	Off white	122-124 m	2:1:1, E or A	59.43	6.25	3.30	4.59	59.82	6.40	3.04	4.71
[(1) <sub>2</sub> -K] <sup>+8,(H8)<sub>2</sub></sup> -	Light brown <sup>d</sup>	84-85 m	2:1:2, Ea	59.75	6.09	4.26	3.96	58.64	6.03	4.11	3.90

<sup>a</sup> Key: m, melting; m,d, melting with decomposition; t, transition. Some complexes melt twice (see table). <sup>b</sup> Key: A, acetone; Aa, acetone-water (98:2); E, absolute ethanol; and Ea, ethanol-water (90:10). <sup>c</sup> This compound is produced along with [(1)<sub>2</sub>-K]<sup>+1,H1</sup>- (see text and ref 16). <sup>d</sup> Solvent from the reaction mixture was evaporated at room temperature until the crystals appeared. <sup>e</sup> 1<sup>-</sup>, 2<sup>-</sup>, ... stand for the chelating anion, L<sup>-</sup>, obtained from the deprotonation of H1, H2, ... <sup>f</sup> ac = acetone.

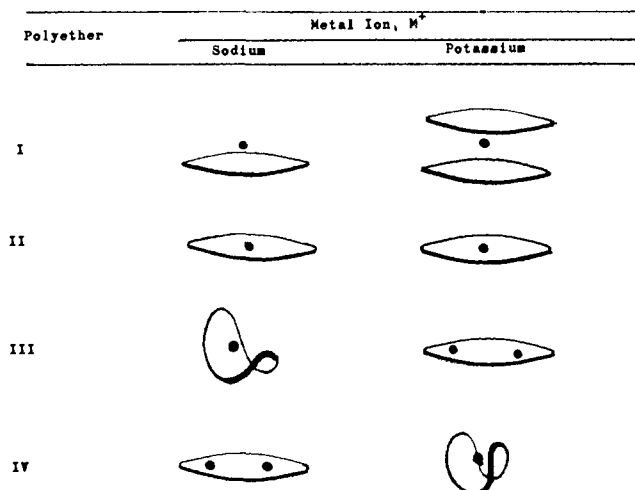


Figure 2. Schematic representation showing stoichiometry of polyether-MX complexes and the conformation of the polyether in the lattice. Solid dot stands for metal ion and the loop for polyether.

ported by other investigators, included for the sake of comparison. A generalized picture of these results is shown schematically in Figure 2. The description of all the new complexes is given in Table II.

The following is the discussion on new compounds obtained in this work and on the ones obtained previously<sup>16</sup>; the latter are included after necessary revision and extension in the light of the new experimental observations.

**Discussion on Complexes from MX.** Complexes of metal chlorides could not be isolated for any of the ligands I to IV. The metal bromides invariably failed to produce isolable complexes with IV and gave irreproducible results with III. The complexes of metal iodides with III also did not crystallize every time. The reason for the failure with III and IV will be given later. The failures to isolate the metal chloride complexes can be attributed to (i) the high lattice energy of the salt or a strong M<sup>+</sup>Cl<sup>-</sup> ion association as a consequence of which M<sup>+</sup> fails to cause necessary polarization of the polyether molecule and (ii) the strong hydrogen bonding

between chloride and the media of synthesis (which has to be methanol for metal chlorides), a point which shall be demonstrated subsequently. Other results are as follows. Ligand I with potassium forms a 2:1 complex irrespective of the nature of the anion. Understandably, a sandwich of the metal ion between two molecules of the ligand, as in case of (I)<sub>2</sub>-KI complex,<sup>12</sup> is formed in each case. With sodium, the complexes are 1:1, monohydrated for bromide and iodide but dehydrated for thiocyanate. Apparently, for halides the lattice involves an ion-separated system where the polyether fails to completely dehydrate the metal ion. In the complex (I)-NaI (aq), single-crystal X-ray structural analysis<sup>9</sup> has shown the sodium ion to be 0.75 Å outside the plane of polyether oxygens (see Figure 2), a direct linkage of a water molecule with the metal atom and a hydrogen bonding of the iodide with two symmetry-related water molecules. The dehydrated state of the (I)-NaNCS complex, however, indicates the possibility of a direct linkage between thiocyanate and sodium and some covalent character in the bond.

Ligand II forms 1:1 complexes both with sodium and potassium, monohydrated for potassium halides, dihydrated for sodium halides, and dehydrated for thiocyanates. Potassium (ionic diameter 2.66 Å) and, of course, sodium ion should fit well into the hole (2.6–3.2 Å diameter)<sup>2</sup> of the polyether with anion and water molecules having the possibilities of interacting with the metal ion from axial directions. The thiocyanate complexes show similarities in infrared spectra particularly with regard to a fine splitting of the  $\nu(\text{C}\equiv\text{N})$  frequency around 2055 (KNCS) and 2065 (NaNCS)  $\text{cm}^{-1}$ . This suggests a similarity in the bonding mode of  $\text{NCS}^-$  with  $\text{K}^+$  and  $\text{Na}^+$ . To account for the dehydrated state of both the complexes, polymerization of complex molecules, where  $\text{NCS}^-$  interacts with the metal ions at both ends, may not be ruled out. From moist media, the (II)-NaNCS complex, however, forms a dihydrate and shows a wider splitting of the  $\nu(\text{C}\equiv\text{N})$  frequency (peaks at 2055 and 2083  $\text{cm}^{-1}$ ) and hence a difference of the  $\text{NCS}^-$ - $\text{Na}^+$  linkage. Since  $\nu(\text{OH})$  peaks are sufficiently sharp and intense (3410, 3500, and 3590  $\text{cm}^{-1}$ ) water appears metal coordinated and the lattice an ion-separated system (see below) as for the 18-crown-6-NaNCS complex<sup>14</sup> (18-crown-6 polyether carries no aromatic nucleus and represents only the polyether ring of dibenzo-18-crown-6).

With III, potassium forms bimetallic complexes, but the only complex isolated reproducibly is with KNCS. This complex was reported<sup>1</sup> earlier to be 1:1, but we found it to be 1:2. Single-crystal X-ray structural analysis<sup>11</sup> shows each metal atom interacting equatorially with five oxygen atoms with the middle two oxygen atoms shared by both the metal atoms. Thiocyanate ions approach the metal atoms from both axial directions from the nitrogen end such that each nitrogen bridges between the two metal atoms. Aromatic nuclei belonging to the adjacent molecules in the lattice also approach from axial directions within the bonding distances with metal atoms and, in addition to the part played by the partial covalency of the  $\text{K}^+$ - $\text{NCS}^-$  bond, account for the dehydrated state of the complex.

With III, sodium forms 1:1 complexes. Each complex, regardless of the nature of the anion, is monohydrated. This suggests that metal ion in these com-

plexes is partially wrapped around by the polyether and the water molecule directly interacts with the metal atom from the open side (see Figure 2) to produce an ion-separated system. Peaks due to  $-\text{OH}$  stretch (see below) also suggest metal-coordinated water in the complexes. Under anhydrous conditions, only NaNCS is complexed to produce a bimetallic system, the infrared spectrum of which is strikingly similar to that of the (III)-(KNCS)<sub>2</sub> complex suggesting a structural similarity between the two.

Ligand IV with potassium forms 1:1 so that thiocyanate complex should also involve a wrap around of the metal ion by the polyether as noted for the (IV)-KI complex<sup>9</sup> and the nonactin-KNCS complex.<sup>22</sup> Both of the sodium complexes isolated with IV are bimetallic in which the polyether molecule appears roughly planar for two reasons, *viz.*, (i) in this state of conformation the two metal atoms will not show electrostatic repulsions and (ii) not much energy needs to be spent to change the conformation of the free polyether.

**Hydration of Complexes and Position of Water in the Lattice.** This is an important aspect and determines to what extent the neutral ligand (R) dehydrates the metal ion and renders it capable of permeation through the lipid phase. Using X-ray structural results on some of these complexes as supporting evidence, the infrared spectrum has been used to decide upon this feature. In addition to the position of the  $\nu(\text{OH})$  peaks, which for a particular MX is changed by changing the polyether, the sharpness was considered as an important feature. A water molecule in the complex is considered metal coordinated if relatively sharp absorption bands appear over 3400  $\text{cm}^{-1}$  especially above 3500  $\text{cm}^{-1}$  but anion bonded if the latter is missing and broad peaks or humps appear around and below 3400  $\text{cm}^{-1}$ .

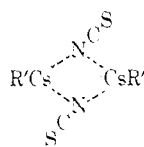
According to this criterion, water molecules are anion bonded only in the (I)<sub>2</sub>-KBr or -KNCS and (IV)-KNCS complexes. Ligand I is half the molecular weight of IV and produces the same effect by sandwiching  $\text{K}^+$  which IV produces by wrapping around. For both polyethers, the KNCS complexes are monohydrated whereas the KI complexes are dehydrated. In view of the molecular weight and the positive charge carried by it, the cation in these complexes possesses a highly decreased charge density. As a consequence of this, the counteranion feels electrostatically destabilized and behaves as an unusually strong proton acceptor. Thiocyanate in this regard appears stronger than iodide and bridges with a molecule of water. This is supported by the isolation of [(IV)-K]<sup>+</sup>NCS<sup>-</sup>(H<sub>5</sub>) which shows broad and strong absorptions at *ca.* 1900 and 2500  $\text{cm}^{-1}$  suggesting a strong hydrogen bond between  $\text{NCS}^-$  and the undissociated organic acid. No such complex can be isolated for iodide.

The pattern of  $-\text{OH}$  stretch peaks for all the (II)-metal halide complexes is roughly the same, each with at least one peak above 3500  $\text{cm}^{-1}$ . In one of them, (II)-NaBr, 2H<sub>2</sub>O, X-ray structural analysis<sup>9</sup> has also shown water to be metal coordinated. Similarly, the  $-\text{OH}$  stretch peaks for all the (III)-metal halide complexes resemble each other and indicate metal coordinated water.

(22) M. Dobler, J. D. Dunitz, and B. T. Kilbourn, *Helv. Chim. Acta*, 52, 2573 (1969).

### Special Remarks on the Metal-Thiocyanate Bond.

For metal thiocyanate complexes of I and II, where polyether oxygens are roughly in a plane and a water molecule can interact with the metal ion from the axial side, the dehydration should be attributed in part to the degree of covalent character in the  $M^+-NCS^-$  bond as already mentioned during the individual discussions on such complexes. This is supported by the fact that  $[I-Na]^+L^-$  and  $[(III)-2M]^{2+}2L^-$  complexes, where some degree of charge transfer between  $M^+$  and  $L^-$  is possible, are constantly dehydrated as against the metal halide complexes of these polyethers which are constantly hydrated. Also, lithium in (I)-LiX (where  $X^- = Br^-$  and  $I^-$ ) complexes are hydrated whereas the corresponding LiNCS complex is dehydrated. Tetramethyldibenzo-18-crown-6 ( $R'$ , a derivative of II where four out of eight methylene groups of the ring carry methyl groups) produces a 1:1 complex with CsNCS but the same dimerizes in the lattice<sup>14</sup> with  $NCS^-$  holding the metal atoms in the fashion



(The repulsive coulombic forces in this complex and in the  $[(III)-2K]^{2+}2NCS^-$  complex, due to short inter-metallic distances, perhaps are not called into play due to the electrostatic stabilization provided to the metal ions by bridging  $NCS^-$  ions.) In fact X-ray crystal structures of various polyether-metal thiocyanate complexes, such as III-(KNCS)<sub>2</sub>,<sup>11</sup> (II)-RbNCS,<sup>23</sup> dicyclohexyl-18-crown-6 (isomer A)-Ba(NCS)<sub>2</sub>,<sup>24</sup> and 18-crown-6-Cs(Rb)NCS<sup>14</sup> show metal-NCS ion pairing at the nitrogen end. Only Na(K)NCS-18-crown-6 complexes constitute exceptions.<sup>14</sup> Here the sodium complex involves a molecule of water to produce an ion-separated system whereas for potassium, the ion-separated system carries disorderd  $NCS^-$  ions.

**General Remarks.** The dimensions of the polyether hole and the metal ion basically control the stoichiometry of the complex and the lattice position of the metal ion with respect to the polyether hole. The spatial position of the polyether in the complex primarily controls the solvation of the metal ion in the latter.

For larger ring polyethers, III and IV, the reaction energy becomes favorable either because of the close approach of the polyether oxygens due to the flexibility of the polyether ring or because of the interaction of two metal ions with a molecule of the latter. However, IV invariably failed to produce isolable metal bromide complexes whereas isolation of metal halide complexes is not reproducible with III. This may be attributed to the high lattice energy of the salt or the ion association between  $M^+$  and  $X^-$  by virtue of which the metal ion tends to be pulled out of the polyether ring as in the complex (II)-NaBr, 2H<sub>2</sub>O.<sup>9</sup> The effect is, obviously, pronounced for large ring polyethers where any water in the synthesis medium aids in the breaking of metal-polyether bonds.

Superimposed on the above given effects can be the effect of hydrogen bonding between  $X^-$  and the solvent

medium as evidenced by the following two observations: (i) potassium halides and thiocyanate are all solubilized in methanol by I but the ease of isolation of the complexes decreases in the order  $NCS^-$ ,  $Br^-$ , and  $Cl^-$  (the last is not isolable at all) which is also the order of their basicity; (ii) if the solvent, methanol, is meticulously dehydrated and crystallization is carried out over anhydrous calcium chloride then the complex  $[(I)_2-K]^+Br^-$  crystallizes out with a molecule of methanol (instead of water) which shows only one strong band from 3100 to 3300  $cm^{-1}$  so that hydrogen bonding of bromide with methanol is evident.

Hydrated complexes can be isolated using anhydrous reactants whereas anhydrous ones appear even when water is deliberately added to the synthesis medium so that the composition of the complexes is unchanged. (II)-NaNCS is an exceptional case for which the dihydrated complex is also obtained from moist solution.

In conclusion, potassium ion is easily dehydrated and shows a high coordination number so as to produce dehydrated complexes with a low charge density on the lipid cationic part. Sodium, on the other hand, is difficultly dehydrated and exhibits a comparatively lower coordination number so that the sodium complexes are usually hydrated and possess high charge density at the cation. Obviously, complexes of potassium should be more acceptable to a lipid phase compared to the sodium products. Parallel effect *in vivo* should contribute toward the preferential uptake of potassium over sodium by the cell membranes.

**Discussion on Complexes from ML.** The results obtained so far with polyethers I to III indicate that the stoichiometries of the complexes are comparable to those from MX. The point of difference is that the chelating anion,  $L^-$ , more effectively dehydrates  $M^+$  compared to  $X^-$  including  $NCS^-$  so that even sodium is dehydrated in complexes with I and III. For II, where complexes of sodium and potassium are constantly monohydrated,  $M^+$  makes an easy fit into the hole of polyether and water is able to interact with it from the vacant axial direction.

With I, sodium complexes are 1:1 whereas those of potassium 2:1. The latter may not be due to the size factor of the cation because thallos (2,4-dinitrophenolate) with I forms a dehydrated 1:1 complex (like sodium) although the ionic radii of potassium and thallos are approximately equal. The complexes of sodium are dehydrated because the metal ion is sandwiched with the chelating anion,  $L^-$ , on one side and the polyether molecule on the other. The sandwiches of potassium involve polyethers on both the sides and the anions in this case are usually conjugated either with a molecule of water or with one or two molecules of HL. This stabilization of  $L^-$  through homo- or heteroconjugation is necessary because of the decreased charge density of the lipid cation as also explained earlier. Homoconjugation takes place even if no free HL is used in the reaction mixture presumably because in the aprotic media  $L^-$  disproportionates to produce HL. Thus,  $[(I)_2-K]^+$  (2-nitrophenolate, 2-nitrophenol)<sup>-</sup> crystallizes out when potassium *o*-nitrophenolate and I alone are used in the proportion 1:2 and especially 1:1. For potassium salts of substituted benzoic acids,  $[L,HL]^-$  and  $[L,(HL)_2]^-$  homoconjugates are produced in addition to  $[L,aq]^-$  so that the products

(23) D. Bright and M. R. Truter, *Nature (London)*, 225, 176 (1970).

(24) N. K. Dalley, D. E. Smith, R. M. Izatt, and J. J. Christensen, *J. Chem. Soc., Chem. Commun.*, 90 (1972).

could not be isolated in pure form until (see below) R, ML, and HL combinations were systematically tried in EtOH as well as EtOH-H<sub>2</sub>O.

With III, both sodium and potassium form dehydrated bimetallic complexes. A single-crystal X-ray structural analysis of one of them, [(III)-2Na]<sup>2+</sup> (2-nitrophenolate)<sub>2</sub><sup>-</sup>, is in progress. According to preliminary information each sodium ion is equatorially chelated by four polyether oxygens whereas the two nitrophenolate ions bridge between the adjacent molecules of the complex in the lattice.

#### Discussion on Results from R, ML, and HL Systems.

In the foregoing studies (I)-KL reactions failed to produce well-defined products which is attributed in most cases to the formation of various homo- and heteroconjugated anions in the reaction. The isolation of compounds containing such anions is of value, however, to the understanding of the permeation mechanism of weak acid uncouplers (HL) of oxidative phosphorylation.<sup>25</sup> For this reason, reactions of ML with R were tried in the presence of varying amounts of parent acid, HL, and water so that the conditions are maintained for the crystallization of different anion conjugates.

Work using HL was carried out using polyethers I to III with sodium and potassium salts of H1 to H8 but compounds of definite composition were only isolated for I-KL systems. The other five combinations, *viz.*, (II)-, (III)-KL, and (I to III)-NaL, which produced well-defined products without the use of HL, produced gums in these experiments suggesting the existence of only weak homoconjugates in solution. This is probably for two reasons: (i) the charge density of the lipid cation, [R-M<sub>n</sub>]<sup>n+</sup>, produced in the quoted five combinations is high compared to [(I)<sub>2</sub>-K]<sup>+</sup> because of the high charge/molecular weight ratio and (ii) L<sup>-</sup> in the gum-forming cases is being directly influenced by M<sup>+</sup> in contrast to the [(I)<sub>2</sub>-K]<sup>+</sup>L<sup>-</sup> sandwiches. Due to both reasons L<sup>-</sup> is not quite allowed to become stabilized by conjugation with HL or water, although, weak L<sup>-</sup>-HL (water) interactions are not ruled out in these cases as evident from the conversion of reactants into gums.

The compounds obtained from these experiments are constituted of the cation [(I)<sub>2</sub>-K]<sup>+</sup> with any of the four anions, L<sup>-</sup>, L,aq<sup>-</sup>, L,HL<sup>-</sup>, and L,(HL)<sub>2</sub><sup>-</sup>. Since the electrostatic attraction of this cation for any L<sup>-</sup> would be very small, the latter is left free to make its independent interactions in solution. Using this cation as counterion, we are developing this isolation technique for the study of conjugation of various organic anions—a phenomenon of importance to natural systems.

With [(I)<sub>2</sub>-K]<sup>+</sup> as the cation, homoconjugates, [L, (HL)<sub>n</sub>]<sup>-</sup>, with *n* exceeding 2 could not be isolated from ethanol at all the concentrations of HL and water. For bare K<sup>+</sup>, even KL,HL species are not isolable<sup>26</sup> from EtOH or EtOH-H<sub>2</sub>O using H3 and H8 as HL. This shows that the homoconjugation of the anion of a weak organic acid is greatly influenced by the nature of the cation—particularly its charge density, in contrast to previous views.<sup>27</sup> It is also influenced by the molecular structure and the electronic configuration of the

anion, L<sup>-</sup>. Thus only the anions of H4 and H6 can be isolated in the unconjugated state. Unconjugated and heteroconjugated, [L,aq]<sup>-</sup>, anions of H8 are not isolable. Anions of H1, H2, H4, and H6 do not produce isolable *n* = 2 homoconjugates.

The nature of the medium is evidently also a factor. Thus, *n* = 2 homoconjugates of H5, H7, and H8 (all substituted benzoic acids) crystallize out only from nearly aqueous media, *i.e.*, after ethanol from EtOH-H<sub>2</sub>O has nearly been expelled by evaporation at room temperature. However, using ML and HL in 1:1 proportion, the first homoconjugate, [L,HL]<sup>-</sup>, in these cases is most readily isolated in the absence of water as if the latter decreases the concentration of L<sup>-</sup> in the reaction mixture by forming [L,aq]<sup>-</sup> (as a consequence of which [L,(HL)<sub>2</sub>]<sup>-</sup> is also formed); L<sup>-</sup> is apparently itself strong enough to depolymerize (HL)<sub>n</sub> for use in homoconjugation. For the production of [L,(HL)<sub>2</sub>]<sup>-</sup>, using ML and HL in ratio 1:2, the presence of water is a necessity as if [L,HL]<sup>-</sup> produced as an intermediate is electrostatically not strong enough to depolymerize the rest of (HL)<sub>n</sub> until water is present in the medium. For the *n* = 2 homoconjugate of H3, however, the presence of water is not that critical. For L<sup>-</sup> of H1, H2, H4, and H6, for which no *n* = 2 homoconjugate is formed, the presence of water in the medium is immaterial so that [L,HL]<sup>-</sup> is produced both from EtOH and EtOH-H<sub>2</sub>O.

**Infrared Spectra.** Except for [6,H6]<sup>-</sup> for which there is a distinct peak at 1640 cm<sup>-1</sup>, the [L,HL]<sup>-</sup> species for carboxy acids in general exhibit only a C=O stretching band whereas compounds containing [L,aq]<sup>-</sup> and [L,(HL)<sub>2</sub>]<sup>-</sup> exhibit broad and intense peaks of the usual type. Thus, [5,H5]<sup>-</sup> and [8,H8]<sup>-</sup> show a shoulder at about 1680 cm<sup>-1</sup> whereas [5,(H5)<sub>2</sub>]<sup>-</sup> and [8,(H8)<sub>2</sub>]<sup>-</sup> give intense peaks at about 1655 cm<sup>-1</sup>. Similarly, [7,H7]<sup>-</sup> and [7,(H7)<sub>2</sub>]<sup>-</sup> give a shoulder and a peak, respectively, at about 1715 cm<sup>-1</sup>.

Except for [5,aq]<sup>-</sup> for which there are two distinct peaks due to ν(OH) at 3380 and 3490 cm<sup>-1</sup>, the [L,aq]<sup>-</sup> species in general have shown an assemblage of broad peaks which collectively show up as a broad and intense band from 3200 to 3500 cm<sup>-1</sup> unlike the compounds containing [L,HL]<sup>-</sup> and [L,(HL)<sub>2</sub>]<sup>-</sup>.

Broad bands at about 1900 and 2500 cm<sup>-1</sup>, which are attributed to intermolecular hydrogen bonding, are visible only for compounds containing [L,(HL)<sub>2</sub>]<sup>-</sup> because the same involve two such bonds but not for [L,aq]<sup>-</sup> and [L,HL]<sup>-</sup> species because of a single bond in high molecular weight compounds. The compound containing [3,(H3)<sub>2</sub>]<sup>-</sup>, however, does not show such bands. The [L,HL]<sup>-</sup> species in general are distinguished from [L,aq]<sup>-</sup> and [L,(HL)<sub>2</sub>]<sup>-</sup> species from their abnormal spectrum in the 750-950 cm<sup>-1</sup> region.<sup>17</sup>

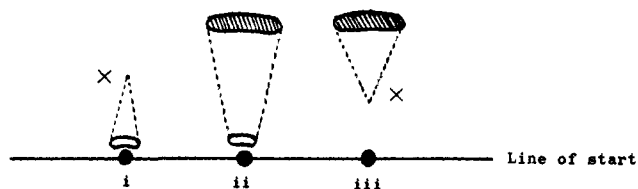
Existence also of [L,aq]<sup>-</sup> and [L,(HL)<sub>2</sub>]<sup>-</sup> possibly explains why physical data taken on bilayers in the presence of weak organic acid uncouplers (HL) of oxidative phosphorylation are not explainable even after considering (for H3) L<sup>-</sup> and [L,HL]<sup>-</sup> as the permeating species.<sup>25</sup>

**Discussion on Competitive Isolation of NaX and KX Complexes.** Table III shows the results of isolation studies with polyethers I to IV where sodium and potassium iodides or thiocyanates were taken as a mixture in solution. Results show that I behaves non-

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**Figure 3.** Schematic representation of the salt zone obtained in the presence of a polyether on a circular chromatogram: (i) slow zone with a forward tail due to weak complexation, (ii) fast zone of the salt in the complex connected to a slow zone of uncomplexed salt by a tail of the salt obtained by the continuous decomposition of the complex during development, and (iii) fast zone of the complex with a tail attributed to slow decomposition of the complex during development.

**Table III.** Showing the Results of Competitive Isolation of Sodium and Potassium Complexes with I to IV

Polyether	Na and K taken as	Reaction mixture R:Na:K	Complex isolated (preferred)
I	Iodides	2:1:1	Both (—)
		2:1:2	K
II	Thiocyanates	2:1:1	Both (—)
	Iodides	1:1:1	Both (Na)
III	Thiocyanates	1:1:1	K
	Iodides	1:1:1	Na
IV	Thiocyanates	1:1:1	Both (K)
	Iodides	1:1:1	K
		1:2:1	Na
		1:1:1	Both (K)
		1:2:1	Both (—)

**Table IV.** Showing Paper Chromatographic Migration of Some Inorganic Salts in the Presence of Polyethers I-IV: Developer, Ethanol; Method of Development, Radial; Time of Development, 2-3 hr

Salt (MX)	$R_f$ value of control <sup>a</sup>	Chromatographic mobility in presence of <sup>b,c</sup>				Stability sequence, decreasing order
		I	II	III	IV	
KBr	4	5, (45)T-74	(62)T-77	5-T(33), 79	5-T-79	II, IV, I, III
KI	13	15-T-82	(10)T-80	6-T-63	(5)T-79	II, IV $\approx$ I, III
KNCS	7	24-T-85	27-T-88	16-T(88)	(17)T-86	IV, II $\approx$ I, III
NaI	22	25-T-74	(52)T-74	26-T(50), 81	22-T(48), 75	II, I, III, IV
NaNCS	14	32-T-84	(35)T-87	34-T(87)	33-T(54)	II, I, III, IV

<sup>a</sup>  $R_f$  values are multiplied by 100 throughout the table for convenience. <sup>b</sup> Amounts of polyethers spotted are as follows (R:MX): I 1:1 with sodium, 2:1 with potassium; II 1:1 both with sodium and potassium; III 1:1 with sodium, 1:2 with potassium; IV 1:2 with sodium, 1:1 with potassium. <sup>c</sup>  $R_f$  value of point "X" (Figure 3(i) and 3(iii)) is shown in parentheses. Migration notations given in table are as follows: 34-T(87) describes the salt distribution as in Figure 3(i); it reads, the  $R_f$  value of the spot is 34 and it tails forward up to  $R_f$  87; 15-T-82 describes the salt distribution as in Figure 3(ii); it reads, two spots at  $R_f$  values 15 and 82 which are connected by a tail. The italic spot is intense. The tail can be discontinuous to produce a case such as 5, (45)T-74. It reads, the slow zone is compact but the fast tails back up to  $R_f$  value 45; (45)T-82: this describes the salt distribution as in Figure 3(iii); it reads, the spot is at  $R_f$  value 82 and it tails back up to  $R_f$  value 45.

selectively but selectively crystallizes the metal present in excess. From an equimolecular mixture the strongly solvated sodium ion also complexes out as if the reaction energy available from the formation of longer ten (2.78–2.96 Å) K–O bonds<sup>12</sup> is nearly approached by the one obtained from the creation of five shorter (2.35–2.43 Å) Na–O bonds.<sup>9</sup>

Polyether IV ordinarily crystallizes potassium out in the presence of sodium but exhibits preference for sodium when the latter is present in excess, a feature attributed to the molecular flexibility of the ligand which is in contrast to the behavior of Lehn's three-dimensional heteroatom ligands.<sup>28</sup> In experiments with IV, the effect of the anion is also detectable but the same is striking in the results with II and III. Thus, both of

(28) B. Dietrich, J. M. Lehn, and J. P. Sauvage, *Tetrahedron Lett.*, 2885, 2888 (1969).

these polyethers preferentially crystallize out potassium when counteranion is NCS<sup>−</sup> but sodium when the anion is iodide.

The rejection of one salt in the presence of another by polyethers is also shown for ML salts. Thus, Na8 is rejected by all the polyethers in preference to K8 in ethanol as well as acetone media. Conductivity measurements of these two salts in both the media show the sodium salt to be more highly ion associated. This agrees with our earlier conclusion that sodium is more "polarophilic" than potassium. Such effects can be generalized for natural systems and should significantly account for a greater resistance of sodium against complexation with natural carriers and its accumulation outside the cell membrane.

**Discussion of Paper Chromatographic Solution Studies.** To compare the interactions of different polyethers with a particular MX, the migration of the latter spotted as a mixture with each polyether was compared with that of the control (pure MX). From the viewpoint of polarity, the migration of the salt, the complex, and the polyether should increase in this order so that an enhancement of migration of MX caused by the presence of the polyether is a qualitative measure of the solution stability of the system R–MX. Paper chromatographic results have shown R–MX interactions for all the systems included in Table I. Important results are shown in Table IV.

Due to the dissociation of the complex in solution, however, either a tailing zone (Figure 3(i) and 3(iii)) of

the salt or two zones connected by a tail (Figure 3(ii)) are obtained. For systems forming increasingly stable complexes, salt on the paper appears in the order i to iii.

For systems involving two different polyethers the comparison of  $R_f$  values of the fast zones (which are given in Table IV) has no significance for the purpose of comparing the solution stabilities because it is difficult to say to what extent the difference is due to the difference of stabilities rather than to the species being different. For similar patterns of salt distribution on the paper for two different complexes, the higher  $R_f$  value of the point "X" in cases i and iii (Figure 3) and more of the salt in the fast zone, compared to the one in the slow zone for case ii, are indications of greater solution stability of the complex.

Although isolation experiments failed, both sodium



and potassium bromides show interaction with IV in ethanolic solution; potassium, which is relatively easily dehydrated, appears more strongly complexed. Furthermore, the stabilities of the KBr complexes decrease in the order II, IV, I, and III (see Table IV) which is in agreement with the stability order of KCl complexes with these polyethers in methanol as found potentiometrically<sup>8</sup> (where cyclohexyl-15-crown-5 has been used in place of I). However, the fact, that (I)<sub>2</sub>-KBr complex but not (IV)-KBr can be isolated shows that the isolation possibility of a complex is not necessarily related to its solution stability. (See the stability order above.) Isolation of only the (II)-KNCS complex

from a 1:1:1 reaction mixture of II, KNCS, and NaNCS (see Table II) from ethanol also suggests this because the differences in R-MX interaction forces for (II)-KNCS and (II)-NaNCS systems, as found paper chromatographically, is only marginal.

**Acknowledgments.** The author is grateful to the late Professor Sir Ronald Nyholm under whose guidance and inspiration the earlier part of this work was started, to Professor M. R. Truter who provided necessary help and facilities during that work, and to Professor M. M. Bokadia who provided facilities to work in this department for the execution of the later part of this work.

## Stereoelectronic Properties of Metalloenzymes. I. A Comparison of the Coordination of Copper(II) in Galactose Oxidase and a Model System, *N,N'*-Ethylenebis(trifluoroacetylacetoniminato)copper(II)

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**Abstract:** The complex, *N,N'*-ethylenebis(trifluoroacetylacetoniminato)copper(II), is proposed as a model of the equatorial coordination of the copper(II) in galactose oxidase. The spin Hamiltonian parameters of galactose oxidase ( $g_{zz} = 2.058$ ,  $g_{yy} = 2.048$ ,  $g_{xx} = 2.273$ ,  $A_{zz} = 28.8$  G,  $A_{yy} = 30.1$  G, and  $A_{xx} = 176.5$  G) are quite similar to the proposed model ( $g_{zz} = 2.0451$ ,  $g_{yy} = 2.0349$ ,  $g_{xx} = 2.2043$ ,  $A_{zz} = 30.7$  G,  $A_{yy} = 31.6$  G, and  $A_{xx} = 194.5$  G) differing only in the magnitude of  $g_{zz}$  and  $A_{zz}$ . This difference is ascribed to the presence of a strong  $\pi$ -bonding axial ligand in galactose oxidase. A calculation of the copper d-d electronic transitions in both model and enzyme based upon the observed esr parameters is in good qualitative agreement with the optical spectra. This agreement suggests both that the model is an appropriate one for galactose oxidase and that the assignment of the spin parameters for each system is correct. Based upon comparisons to the known copper ligation in the model system, possible features of the copper-protein complex are suggested.

Galactose oxidase (galactose, O<sub>2</sub> oxidoreductase EC 1.1.3.9, hereafter referred to as GOase) is a copper-containing enzyme which catalyzes the conversion of primary alcohols to the corresponding aldehydes concomitant with the reduction of O<sub>2</sub> to H<sub>2</sub>O<sub>2</sub>.<sup>2-9</sup> The molecular weight of this enzyme has been established at 68,000  $\pm$  3000 daltons with one copper atom per molecule.<sup>8,9</sup> The nature of the copper(II) site and the

mechanism of the enzymatic reaction<sup>6,7,10</sup> have received some attention, but until recently no definitive information has been obtained. Ettinger and Kosman<sup>11</sup> have reported a detailed CD study of the enzyme in the presence of its substrates and products. Also, the optical spectrum of the enzyme from 350 to 950 nm has been correlated with the CD spectrum.<sup>12</sup> The electron spin resonance spectrum of GOase was reported by Blumberg,<sup>13</sup> but, as previously noted,<sup>14</sup> the conditions of his experiment, when duplicated in our laboratory, inactivate the enzyme. The electron spin resonance spectrum of GOase has recently been employed in this laboratory to establish the nonexistence of a cuprous (Cu<sup>+</sup>) intermediate in the enzymatic reaction.<sup>14</sup>

Copper-containing proteins, since initial studies were

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